Vitamin Supplementation Effects on Homocysteine and Psychological Functioning

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation
with distinction in Psychology in the undergraduate colleges
of The Ohio State University

by

Marcy Camilla P. Trinidad

The Ohio State University
December 2005

Project Adviser: Dr. Charles Emery, Department of Psychology

Abstract

Elevated homocysteine levels are associated with increased risk of heart disease. One possible strategy to reduce risk of heart disease is through reduction of homocysteine with vitamin B. Eighty middle-aged men and women participated in a four-week study examining vitamin supplementation effects. Pre-test measures consisted of the Beck Depression Inventory, Revised Life Orientation Test, Cohen's Perceived Stress Scale, and an assessment of homocysteine. Post-test measures included the same battery of tests as well as the Profile of Mood States. Participants were randomly assigned to receive either the self-administered supplement consisting of 0.4 mg folate, 0.4 mg B_{12}, and 10 mg B_6 or a placebo. Data were analyzed with repeated measures analysis of variance with time (pre-test v. post-test) as a within-subjects factor and condition (vitamin v. placebo) as a between-subjects factor. With an alpha level of 0.05, no statistically significant effect of supplementation was found for depression, F (1, 80) = 2.27, p = 0.14 or homocysteine, F (1, 80) = 0.80, p = 0.37.

Vitamin Supplementation Effects on Homocysteine and Psychological Functioning

High rates of coronary heart disease (CHD) have piqued researchers' interests in examining mechanisms within the body that contribute to CHD development. According to recent statistics from the American Heart Association (2005), 13 million people in the US in 2005 were diagnosed with heart disease and it is the primary cause of death and permanent premature disability. Also, $393.5 billion was the 2005 estimate for medical expenses and lost productivity due to heart disease. Because of the high cost of CHD for society, present and future research should focus on prevention of heart disease development.

Interest in metabolic factors affecting the heart was initiated with the observation that individuals with malfunctioning metabolism had higher homocysteine (Hcy) levels and higher rates of atherosclerosis and premature death than the normal population (McCully, 1969). Following this observation, several studies investigated possible relationships between Hcy and other known risk factors for CHD development, including older age and elevated blood pressure (Schaefer, 2002; Nygard et al., 1995). The role of Hcy in heart problems like myocardial infarction (MI) and stroke was further investigated (Nygard et al., 1997). It was hypothesized that elevated Hcy levels increase vulnerability to atherosclerosis, which is the first step in developing heart disease. Although the actual mechanism of Hcy increasing CHD risk is unclear, past research has shown that Hcy and CHD risk are positively correlated (Zylberstein et al., 2004; Whincup et al., 1999). As a result, factors affecting Hcy were investigated with a few researchers focusing on the relationship between B vitamins and Hcy (Hvas, Ellegaard, & Nexo, 2001; Lee et al., 2004). The general finding has been that deficiency of B vitamins, specifically B_{12} and folic acid, lead to increases in Hcy. This increase in Hcy is due to the body's inability to

metabolize Hcy. Paul, McDonnell, and Kelly (2004) established vitamin B as one of the primary factors in Hcy metabolism.

In addition to the previously found influence of vitamin deficiency on physical health, Penninx et al. (2000) found that vitamin B_{12} deficiency is associated with a two-fold increased risk of severe depression. Baldewicz et al. (2000) also found that vitamin B_{12} is inversely related to self-report measures of distress and clinically rated depressed mood. Lower folate levels were also associated with depression (Fava et al., 1997).

Studies have found that depression is a significant risk factor for CHD morbidity and mortality after MI (Carney & Freedland, 2003). Suls and Bunde (2005) found that anxiety and depression play a role in exacerbating the physical process of development of heart disease. In 2000, Frasure-Smith et al. found that depression was associated with a 3 to 4-fold increase in cardiac mortality. Frasure-Smith and Lespérance (2005) then stated that depression is associated with at least a doubling in risk of mortality and recurrent cardiac events in patients with CHD. However, the causal relationship between depression and CHD is not known due to many possible mechanisms leading to either disease. Despite the inexplicable mechanisms behind the two diseases, there is growing support that depression is associated with increased risk of cardiac events in people with CHD as well as development of CHD in healthy individuals.

Earlier studies have shown that B vitamins, Hcy, and psychological functioning are inter-related. However, the relationship between Hcy and psychological functioning has not been studied extensively. In order to assess the relationship between psychological functioning and Hcy, measures of depression, stress, optimism, and distress were used to define psychological functioning. It was hypothesized that dietary supplementation with B vitamins would improve

psychological health and reduce Hcy. Also, lower psychological distress was hypothesized to be associated with lower Hcy levels.

Methods

Sample

Participant demographics. Eighty local Columbus residents (37 men, 43 women, mean age = 39 years) were recruited through the use of flyers and advertisements directed toward the Ohio State University (OSU) campus and Columbus community. Volunteers were screened for eligibility and healthy individuals were asked to participate in the study. A $300 payment given to participants upon completion of the study was made possible by the NIH.

Eligibility criteria. General health of individuals interested in participating was determined during a brief telephone screening of the volunteers. Men and women taking medication for diabetes, hypertension, or CHD were ineligible to participate. Individuals 30 % over or under their ideal body weight, with renal disease or other metabolic diseases, severely depressed, taking vitamin or herbal supplements were also ineligible. Discontinuation of vitamin or herbal supplements for the duration of the study allowed volunteers to become eligible for the study. In addition to these criteria, women were required to be premenopausal and have normal menstrual cycles. Women who were pregnant, nursing, or taking oral contraceptives within the past six months were ineligible to participate.

Design

A 2 (vitamin v. placebo) x 2 (pre-test v. post-test) factorial design was used to measure the influence of vitamin supplementation on Hcy levels during the month-long intervention.

Apparatus

Physiological measures. Hcy was measured using high performance liquid chromatography (HPLC) with fluorescence. Serum folate was measured using the ADVIA Centaur System. Serum B_{12} was measured with the Magnetic Separation Immunoassay. Plasma B_6 was measured using HPLC with electrochemical detection. Total cholesterol, triglyceride levels, and high and low density lipoproteins were also measured. Heart rate and blood pressure data were collected as well.

Psychological measures. Several scales were used to evaluate psychological functioning. The Beck Depression Inventory (BDI) is a measure of current affective, cognitive, and physiological symptoms of depression for both normal and clinical populations (Beck, Steer, & Garbin, 1988). Cohen's Perceived Stress Scale (PSS-10) is useful in measuring the degree to which individuals perceive the environment and their experiences as stressful. The PSS-10 has shown sensitivity to ongoing life circumstances, and is a valid measure of vulnerability to stress (Cohen, 1988). The Revised Life Orientation Test (LOT-R) is an internally consistent measure of an individual's general outlook on life and level of optimism (Scheier, Carver, & Bridges, 1994). The Profile of Mood States (POMS) measures total mood disturbance consisting of different subscales that measure changes in levels of anxiety, depression, anger, vigor, fatigue, and confusion (McNair, Lorr, & Droppleman, 1971). All measures for the study have been shown to have acceptable to high reliability and validity in indicating psychological health.

Procedure

Pre-test. All prospective participants completed a telephone screening regarding information about the participant's diet, vitamin or herbal intake, and medication. If eligibility criteria were met, a meeting was scheduled to begin participation in the study. Directions and a

map of OSU, along with contact information and a parking voucher were mailed to the participant. The participant met with the investigator and on-site nutritionist on the scheduled date who informed him or her about the risks and benefits of participation in the study. The participant was randomly assigned to either the vitamin or placebo condition after consenting to participate. At that time, the BDI, LOT-R, and PSS-10 were completed. Blood was drawn to measure total Hcy. The vitamin or placebo supplements were given to the participant and a follow-up phone call before the next session was used as a reminder for regular supplement intake. Participants were required to take the supplements daily for four weeks. Vitamin group participants were given supplements consisting of 0.4 mg folate, 0.4 mg vitamin B_{12}, and 10 mg vitamin B_6. Placebo group participants received supplements consisting of cornstarch that were similar to the vitamins in size and tendency to discolor urine. Discoloration of urine is one of the effects of vitamin supplements and was a necessary effect for placebo supplements to duplicate. Participants were instructed to hold a 12-hour fast before the final session.

Post-test. The participant met with the investigator and nutritionist for the post-test four weeks after the initial session. The BDI, LOT-R, PSS-10, and POMS were completed during the final session. Blood was drawn to measure total Hcy. The participant was dismissed and mailed $300 for completion of the study.

Data Analyses

Correlational analyses were performed to assess the relationship between B vitamins, Hcy, and psychological functioning. Repeated measures analysis of variance (ANOVA) was used to assess change in Hcy, psychological functioning, and B vitamins over time. For the repeated measures ANOVA, time (pre-test v. post-test) was a within-subject variable and condition (vitamin v. placebo) was a between subject variable.

Results

Correlational analyses indicated a modest correlation ($r = 0.24$, $p = 0.03$) between pre-test BDI scores and Hcy of all participants. LOT-R and PSS-10 scores were not associated with Hcy. Simple descriptive analyses showed that participants with elevated depression scores (i.e. BDI scores of 10 or greater) had a mean Hcy level of $M_D = 10.31$ while non-depressed participants had a mean Hcy level of $M_N = 7.98$ as shown in Figure 1. Analysis of variance (ANOVA) indicated that the difference in pre-test Hcy levels of depressed and non-depressed participants was significant, $F(1, 80) = 6.29$, $p = 0.01$. Pre-test ANOVA of Hcy levels indicated no difference between vitamin group participants (mean Hcy level of $M_V = 8.15$) and placebo group participants (mean Hcy level of $M_P = 8.72$) as shown in Table 1, $F(1, 80) = 0.55$, $p = 0.46$. Pre-test BDI scores were then analyzed and results showed no difference in BDI scores based on condition. Vitamin group participants had a mean BDI score of $M_V = 5.85$ while placebo group participants had a mean of $M_P = 5.68$, as shown in Table 1 and Figure 2, $F(1, 80) = 0.02$, $p = 0.90$. Pre-test ANOVA of folate levels revealed no difference between vitamin group participants (mean folate level of $M_V = 19.57$) and placebo group participants (mean folate level of $M_P = 18.85$) as shown in Table 1, $F(1, 80) = 0.35$, $p = 0.56$. Pre-test ANOVA of vitamin B_6 and vitamin B_{12} based on condition also revealed no difference between participants' vitamin levels. Refer to Table 1 for means and standard deviations for vitamin B_6 and vitamin B_{12}.

Repeated measures ANOVA for Hcy revealed no interaction of time by condition, $F(1, 80) = 0.80$, $p = 0.37$, but a time main effect for Hcy occurred regardless of condition, $F(1, 80) = 15.34$, $p = 0.00$. Hcy levels of vitamin and placebo group participants decreased over time as shown in Table 1. Note that although Hcy levels of all participants decreased, vitamin group participants' Hcy levels decreased somewhat more than Hcy levels of placebo group participants.

Repeated measures ANOVA for depression scores revealed no effect of time or group, F (1, 80) = 2.27, p = 0.14. As shown in Table 1 and Figure 2, BDI scores of vitamin group participants decreased, but BDI scores of placebo group participants increased. Neither of these changes was statistically significant. Repeated measures ANOVA of folate, vitamin B_6, and vitamin B_{12} between session 1 and session 3 revealed no time by condition interaction. However, time main effects were found for all vitamins: folate, F (1, 80) = 5.67, p = 0.02; vitamin B_{12}, F (1, 80) = 13.72, p = 0.00; and vitamin B_6, F (1, 80) = 20.58, $p < 0.00$. Repeated measures ANOVA with participants divided into depressed and non-depressed groups indicated a time main effect for Hcy, F (1, 80) = 0.14, p = 0.71. Simple effects indicated that Hcy levels of participants with elevated depression scores experienced a somewhat larger reduction in Hcy levels (mean Hcy of M_D at post-test = 7.53) compared to non-depressed participants (mean Hcy of M_N at post-test = 7.82), as shown in Figure 1. Post-test correlational analyses revealed that BDI, LOT-R, and PSS-10 scores were not associated with Hcy. However, POMS scores were found to be moderately associated with Hcy (r = 0.23, p = 0.04).

Discussion

The study was designed to evaluate the effect of dietary supplementation with B vitamins on psychological functioning and Hcy. Another goal of the study was to evaluate the relationship between psychological functioning and Hcy. Although the results were statistically non-significant, the changes observed generally supported the hypotheses.

The association of BDI scores and Hcy levels supports the idea that psychological functioning and Hcy are related. However, after treatment, BDI was no longer associated with Hcy, but POMS was better correlated with Hcy, which suggests that global distress was a better indicator of psychological functioning as it relates to Hcy instead of depression. This finding is

probably due to the fact that POMS measures a wider range of psychological functions than the BDI. Unfortunately, the POMS was not administered at baseline. Future studies should consider administering the POMS during pre-test analyses in order to have stronger support for its association with Hcy as a measure of psychological functioning. LOT-R and PSS-10 scores had almost non-existent correlations with Hcy, indicating that optimism and perceived stress were not related to Hcy. Optimism was not expected to respond to the vitamin intervention because it is a generally stable measure of personality functioning. Perceived stress, however, was expected to be responsive to the vitamin intervention. Also, because stress results in physiological manifestations in the body, stress could have a greater effect on Hcy. However, the PSS-10 data indicated that participants were not experiencing significant distress.

Of greatest interest were results indicating a time main effect for Hcy. Two analyses of Hcy were performed and both found that Hcy decreased regardless of condition (vitamin v. placebo) or psychological health (depressed v. not depressed). Although it would have been ideal to find that Hcy decreased only for vitamin group participants, the Hcy decrease observed in all participants is also a favorable result. This could indicate that time alone could decrease Hcy. However, because the vitamin group experienced a somewhat larger reduction in Hcy than the placebo group, this could still be an indication that vitamin supplementation has a stronger effect on Hcy than time alone. Although this was the case, it cannot be concluded that treatment was the cause for this slightly larger decrease, especially since all participants experienced the decrease in Hcy. Additionally, participants with elevated depression scores experienced a slightly larger reduction in Hcy compared to participants with normal BDI scores.

Clinically significant change is usually defined as a meaningful degree of change that resulted from an intervention or treatment (Kazdin, 2003, p. 692). However, any amount of

change may mean something depending on the type of problem, as well as the goals of the treatment. When dealing with health issues, such as the reduction of Hcy, even a small decrease may be important because Hcy has been associated with heart disease development. Interestingly, absence of change is important in that it offers information about stability over time.

Another interesting finding was that of the relationship between vitamin supplementation and psychological functioning defined by BDI scores. Vitamin group participants had decreased BDI scores over time while placebo group participants were found to have increased BDI scores. We can infer that supplementation may improve psychological functioning, but it cannot be confidently concluded that it was vitamin supplementation that led to the improvement in psychological functioning. No conclusions can be made because results were statistically non-significant. Also, BDI scores were not above the cutoff for mild clinical depression, meaning that the participants were generally of normal mental health and any improvements in their psychological functioning may not be clinically meaningful. Although most of the results were statistically non-significant, meaning that the finding or difference cannot be confidently considered as unlikely due to chance, according to Cowles & Davis (2003), "any level of significance is a useful guideline and may provide a filter for making decisions about effects and variables to be further examined" (p. 381).

Due to a small sample size, which was smaller than the recommended sample size to obtain significant statistical power, results were non-significant and therefore, no solid conclusions can be made about the findings. Also, due to lack of clinically depressed participants, possible vitamin effects on psychological health were not as noteworthy as expected. The study also lacked participants with elevated Hcy levels, which could have

contributed to non-significant effects of vitamins on Hcy. Future studies should obtain a larger sample size that includes moderately to severely depressed participants, as well as participants with elevated Hcy levels. It may be that vitamin supplementation has a larger effect on these types of participants. Healthy middle-aged participants may not be as responsive to vitamin effects, in part, because they are less likely to have deficient vitamin levels in the blood. Despite the lack of an extreme clinical sample, meaning that the sample was within the normative range of the variables examined, normative data is still useful as a criterion for determining clinical significance and as a comparison for problem domains (Kazdin, 2003, p. 691).

The overarching goal of this project is to find more efficient and economical ways of reducing heart disease risk. In this particular study, the primary way of reducing heart disease risk that was promoted was reduction of Hcy. However, Hcy is only one of many factors that contribute to heart disease and should not be the primary concern when taking preventative measures against development of heart disease. Other factors such as diet, age, and level of physical activity should also be taken into consideration. The immediate goal of this study was to find possible factors affecting Hcy, specifically psychological functioning and vitamin supplementation. That goal was obtained by the statistical analyses performed. However, no further conclusions about the role of Hcy reduction in heart disease prevention can be made confidently.

References

American Heart Association (2005). *Heart Disease and Stroke Statistics – 2005 Update.* Retrieved July 4, 2005, from http://www.americanheart.org.

Baldewicz, T.T., Goodkin, K., Blaney, N.T., Shor-Posner, G., Kumar, M., Wilkie, F.L., Baum, M.K., & Eisdorfer, C. (2000). Cobalamin level is related to self-reported and clinically rated mood and to syndromal depression in bereaved HIV-1^+ and HIV-1^- homosexual men. *Journal of Psychosomatic Research, 48,* 177-85.

Beck, A., Steer, R., & Garbin, M. (1988). Psychometric properties of the Beck Depression Inventory: 25 years of evaluation. *Clinical Psychology Review, 8,* 77-100.

Carney, R.M. & Freedland, K.E. (2003). Depression, mortality, and medical morbidity in patients with coronary heart disease. *Biological Psychiatry, 54,* 241-7.

Cohen, S. (1988). Perceived stress in a probability sample of the United States. In S. Spacapa & S. Oskamp (Eds.), *The Social Psychology of Health* (p. 31-67). Thousand Oaks, CA: Sage.

Cowles, M. & Davis, C. (2003). On the origins of the .05 level of statistical significance. In A. Kazdin (Ed.), *Methodological Issues and Strategies in Clinical Research* (pp. 379 - 388). Washington DC: American Psychological Association.

Fava, M., Borus, J.S., Alpert, J.E., Nierenberg, A.A., Rosenbaum, J.F., & Bottiglieri, T. (1997). Folate, vitamin B_{12}, and homocysteine in major depressive disorder. *The American Journal of Psychiatry, 154,* 426-28.

Frasure-Smith, N. & Lesperance, F. (2005). Depression and coronary heart disease: Complex synergism of mind, body, and environment. *Current Directions in Psychological Science, 14*, 39-43.

Frasure-Smith, N., Lesperance, F., Gravel, G., Masson, A., Juneau, M., Talajic, M., & Bourassa, M.G. (2000). Social support, depression, and mortality during the first year after myocardial infarction. *Circulation, 101,* 1919-24.

Hvas, A.M., Ellegaard, J., & Nexo, E. (2001). Vitamin B_{12} treatment normalizes metabolic markers but has limited clinical effect: a randomized placebo-controlled study. *Clinical Chemistry, 47,* 1396-404.

Kazdin, A. (2003). Clinical significance: Measuring whether interventions make a difference. In A. Kazdin (Ed.), *Methodological Issues and Strategies in Clinical Research* (pp. 691 – 710). Washington DC: American Psychological Association.

Lee, B.J., Huang, M.C., Chung, L.J., Cheng, C.H., Lin, K.L., Su, K.H., & Huang, Y.C. (2004). Folic acid and vitamin B_{12} are more effective than vitamin B_6 in lowering fasting plasma homocysteine concentration in patients with coronary artery disease. *European Journal of Clinical Nutrition, 58,* 481-7.

McCully, K.S. (1969). Vascular pathology of homocysteinemia: implications for pathogenesis of arteriosclerosis. *American Journal of Pathology, 56,* 111-28.

McNair, D., Lorr, M., & Droppleman, L. (1971). Profile of Mood States Manual. San Diego: Education and Industry Testing Service.

Nygard, O., Vollset, S.E., Refsum, H., Stensvold, I., Tverdal, A., Nordrehaug, J.E., Ueland, M., & Kvale, G. (1995). Total plasma homocysteine and cardiovascular risk profile. The Hordaland homocysteine study. *The Journal of the American Medical Association, 274,* 1526-33.

Nygard, O., Nordrehaug, J.E., Refsum, H., Ueland, P.M., Farstad, M., & Vollset, S.E. (1997). Plasma homocysteine levels and mortality in patients with coronary artery disease. *The New England Journal of Medicine, 337,* 230-6.

Paul, R., McDonnell, A.P., & Kelly, C.B. (2004). Folic acid: neurochemistry, metabolism, and relationship to depression. *Human Psychopharmacology, 19,* 477-88.

Penninx, B.W., Guralnik, J.M., Ferrucci, L., Fried, L.P., Allen, R.H., & Stabler, S.P. (2000). Vitamin B_{12} deficiency and depression in physically disabled older women: epidemiologic evidence from the women's health and aging study. *The American Journal of Psychiatry, 157,* 715-21.

Schaefer, E.J. (2002). Lipoproteins, nutrition, and heart disease. *The American Journal of Clinical Nutrition, 75,* 191-212.

Scheier, M., Carver, C., & Bridges, M. (1994). Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. *Journal of Personality and Social Psychology, 67,* 1063-78.

Suls, J. & Bunde, J. (2005). Anger, anxiety, and depression as risk factors for cardiovascular disease: the problems and implications of overlapping affective dispositions. *Psychological Bulletin, 131,* 260-300.

Whincup, P.H., Refsum, H., Perry, I.J., Morris, R., Walker, M., Lennon, L., Thomson, A., Ueland, P.M., & Ebrahim, S.B. (1999). Serum total homocysteine and coronary heart disease: prospective study in middle-aged men. *Heart, 82,* 448-54.

Zylberstein, D.E., Bengtsson, C., Bjorkelund, C., Landaas, S., Sundh, V., Thelle, D., & Lissner, L. (2004). Serum homocysteine in relation to mortality and morbidity from coronary

heart disease: a 24-year follow-up of the population study of women in Gothenburg. *Circulation, 109,* 601-6.

Table 1

Mean and Standard Deviation of Variables of Interest for

Vitamin and Placebo Group Participants Over Time

	Vitamin				Placebo			
Variables	Pre-test		Post-test		Pre-test		Post-test	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Hcy	8.15	3.70	7.10	2.69	8.72	3.20	8.06	2.99
B_6	22.77	52.96	29.04	51.46	14.58	10.37	28.37	22.68
B_{12}	574.58	197.21	556.58	217.66	632.03	266.34	640.05	262.96
Folate	19.57	5.33	20.62	4.54	18.85	5.30	19.89	4.74
BDI	5.85	5.43	4.56	4.13	5.68	5.96	6.61	7.29
LOT-R	12.41	4.31	10.59	4.04	12.80	4.90	11.73	5.99
PSS-10	13.77	6.82	10.44	5.92	14.00	7.94	12.80	8.01
POMS	n/a		27.62	9.45	n/a		31.88	12.00

Figure Caption

Figure 1. Change in mean Hcy levels of depressed and non-depressed participants.

Figure 2. Change in mean BDI scores of vitamin and placebo group participants over time.

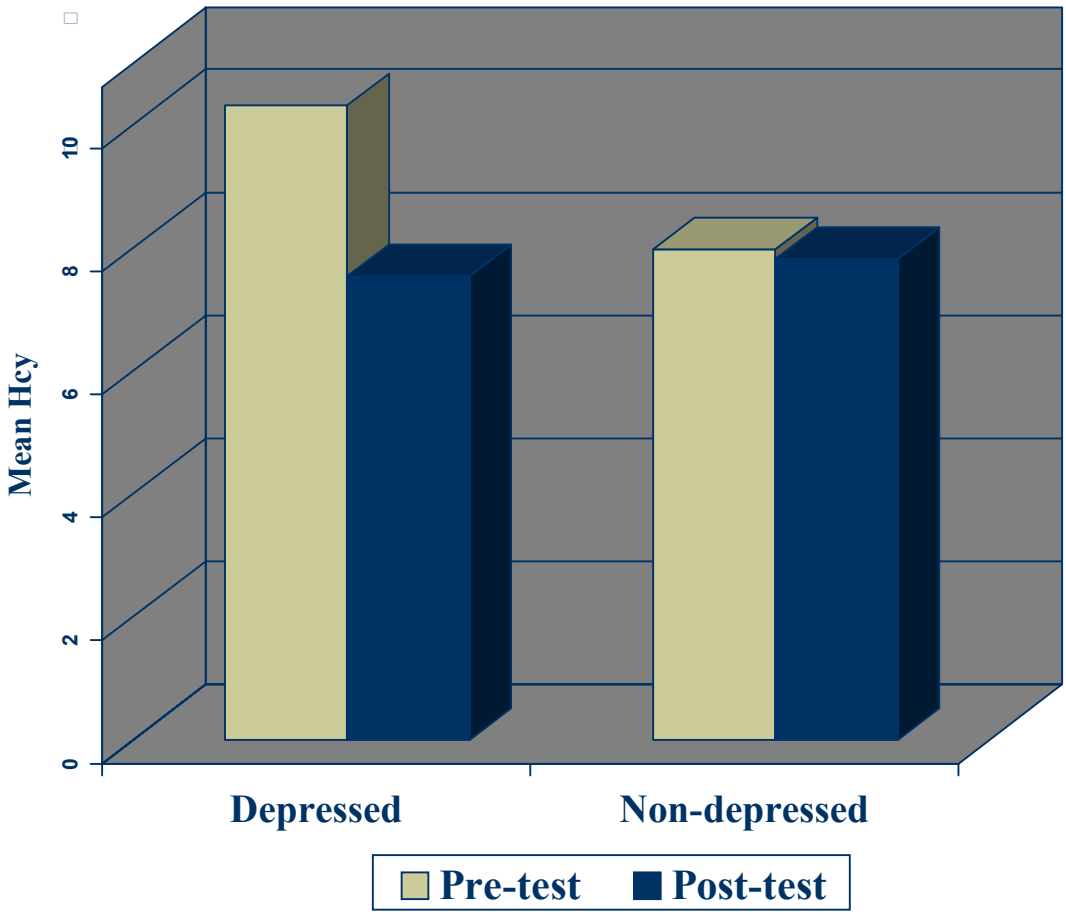

Figure 1. Change in Hcy levels of depressed and non-depressed individuals. ANOVA indicated that pre-test differences in Hcy levels were largely different between participants.

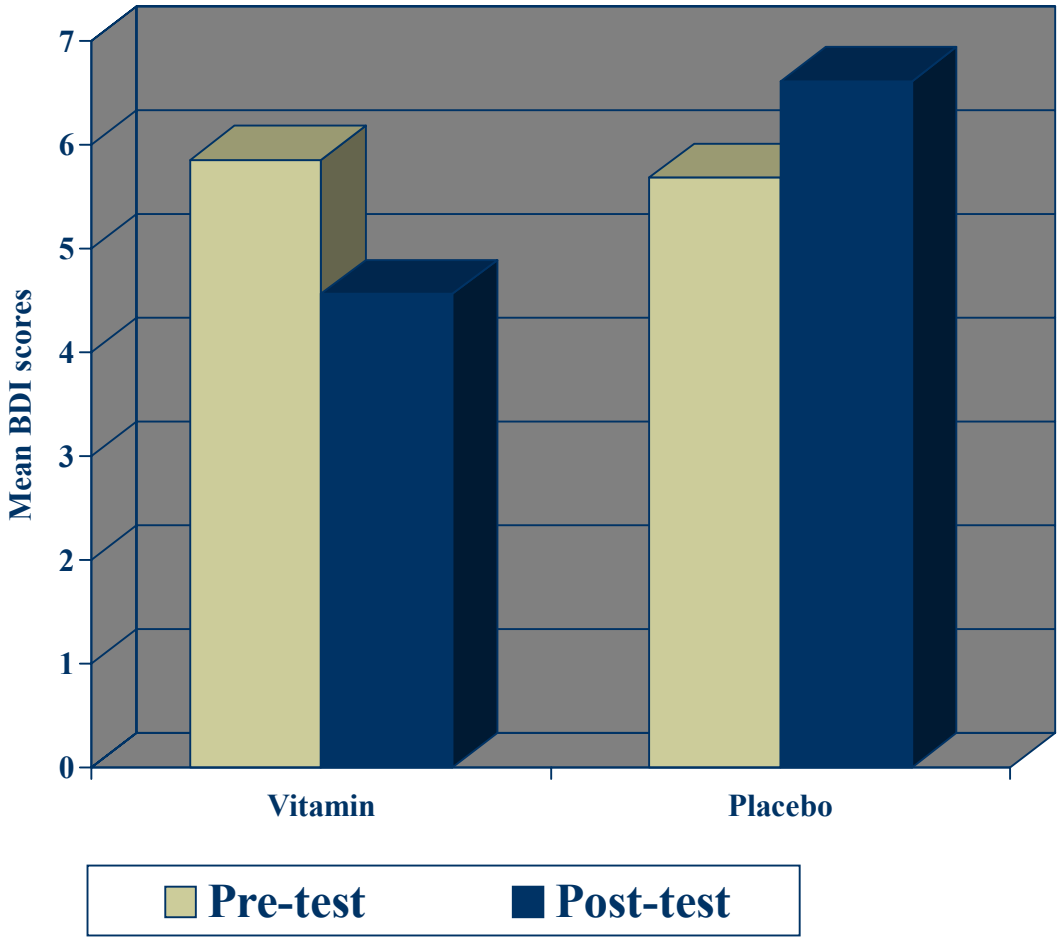

Figure 2. Change in mean BDI scores of vitamin and placebo group participants over time showed that mean BDI scores of vitamin group participants decreased and mean BDI scores of placebo group participants increased.